# CORRESPONDENCE

## Emergence of carbapenem-hydrolysing metallo-β-lactamase VIM-1 in *Pseudomonas aeruginosa* isolates in France

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Recent articles in *CMI* have described the dissemination of metallo-β-lactamase (MBL) genes conferring resistance to carbapenems [1,2]. Carbapenem-hydrolysing β-lactamases identified in *Pseudomonas aeruginosa* have mostly been of the IMP and VIM families [3]. Although VIM-1 was the first determinant described in Europe (in Italy), VIM-2 determinants have spread worldwide and have been reported in isolates causing outbreaks of infection [4]. We wish to report the first isolation in France of *P. aeruginosa* isolates producing the VIM-1 type MBL.

A prospective study was carried out among a collection of 1400 *P. aeruginosa* isolates recovered from 105 French hospitals during 2004–2005. Twenty-three isolates exhibited resistance to ticarcillin, associated with resistance to ceftazidime or imipenem, but only four were recognised as MBL producers using Etest strips for MBL detection (AB Biodisk, Solna, Sweden) [3]. Isolates V4 (Saint Germain-en-Laye) and P0510 (Nantes) were from urine, whereas V115 (Le Mans) and V1236 (Vannes) were from bronchial aspirates. MICs were determined by agar dilution and interpreted as recommended by CLSI guidelines [5], with *P. aeruginosa* VR-143/97 (VIM-1-positive) as a reference strain [6]. All four isolates were resistant to all available antibiotics, except colimycin, fosfomycin, amikacin and aztreonam.

PCR with $bla_{VIM}$- and $bla_{IMP}$-specific primers, followed by sequencing, revealed an identical $bla_{VIM-1}$ gene in each MBL-positive isolate [7]. PCR mapping [8] and sequencing of the flanking regions revealed an integron, In*70.2*, which was identical to that found in the four *P. aeruginosa* isolates described by Lauretti *et al*. [6]. Conjugation experiments [7], using the four VIM-1-producing isolates as donors, failed to yield transconjugants. To search for a possible chromosomal location of the MBL gene, the I–*Ceu*I endonuclease technique was used [9]. The internal $bla_{VIM-1}$ probe hybridised with a very large fragment that co-hybridised with the 16S rRNA probe, indicating a chromosomal location for the $bla_{VIM-1}$ gene, as reported previously for *P. aeruginosa* VR-143/97 [6].

Pulsed-field gel electrophoresis analysis was also performed in order to genotype the four VIM-1-positive isolates [4]. Although V4, V115 and V1236 had been recovered from different hospitals located at least 200 km from each other, these isolates were clonally related, while isolate P0510 belonged to a distinct clone. Pulsed-field gel electrophoresis analysis also indicated that those two pulsotypes were different from that of the *P. aeruginosa* VR143/97 strain from Italy [6].

VIM-1 is the second MBL (after VIM-2) that has been identified as a cause of carbapenem resistance in *P. aeruginosa* isolates in France. In nearby European countries (Greece and Italy), VIM-1 is predominant and has been associated with large outbreaks of multidrug-resistant *P. aeruginosa* or Enterobacteriaceae [3]. In Spain, following the discovery of VIM-2 in *P. aeruginosa*, strains of *Escherichia coli* and *Klebsiella pneumoniae* producing VIM-1 have recently been isolated [3]. Thus, VIM-1 and VIM-2 may coexist in southern Europe in *P. aeruginosa* strains.

Interestingly, the genetic structure surrounding $bla_{VIM-1}$ was identical to that reported previously in Italy [6], which suggests the possibility of a conserved $bla_{VIM-1}$ integron structure in non-clonally related *P. aeruginosa* isolates, as has also been suggested recently by Riccio *et al*. [9].

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## REFERENCES

1. Sader HS, Reis AO, Silbert S, Gales AC. IMPs, VIMs and SPMs: the diversity of metallo-β-lactamases produced by carbapenem-resistant *Pseudomonas aeruginosa* in a Brazilian hospital. *Clin Microbiol Infect* 2005; **11**: 73–76.
2. Viana Vieira V, Lourenço da Fonseca É, Paulo Vicente AC. Metallo-β-lactamases produced by carbapenem-resistant *Pseudomonas aeruginosa* in Brazil. *Clin Microbiol Infect* 2005; **11**: 937.
3. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-β-lactamases: the quiet before the storm? *Clin Microbiol Rev* 2005; **18**: 306–325.
4. Aubron C, Poirel L, Fortineau N, Nicolas P, Collet L, Nordmann P. Nosocomial spread of *Pseudomonas aeruginosa* isolates expressing the metallo-β-lactamase VIM-2 in a haematology unit of a French hospital. *Microb Drug Resist* 2005; **11**: 254–259.
5. Clinical and Laboratory Standards Institute. *Performance standards for antimicrobial susceptibility testing*, 15th informational supplement. M100-S15. Wayne, PA: CLSI, 2005.
6. Lauretti L, Riccio ML, Mazzariol A *et al.* Cloning and characterization of $bla_{VIM}$, a new integron-borne metallo-β-lactamase gene from a *Pseudomonas aeruginosa* clinical isolate. *Antimicrob Agents Chemother* 1999; **43**: 1584–1590.
7. Poirel L, Naas T, Nicolas D *et al*. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. *Antimicrob Agents Chemother* 2000; **44**: 891–897.
8. Lévesque C, Piche L, Larose C, Roy PH. PCR mapping of integrons reveals several novel combinations of resistance genes. *Antimicrob Agents Chemother* 1995; **39**: 185–191.
9. Riccio ML, Pallecchi L, Docquier JD *et al*. Clonal relatedness and conserved integron structures in epidemiologically unrelated *Pseudomonas aeruginosa* strains producing the VIM-1 metallo-β-lactamase from different Italian hospitals. *Antimicrob Agents Chemother* 2005; **49**: 104–110.


## Invasive pneumococcal disease in adults in North-Rhine Westphalia, Germany

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The recent paper in *CMI* by Reinert *et al*. [1] presented interesting information on invasive pneumococcal disease in adults in North-Rhine Westphalia, Germany, which was collected prospectively. It was reported that blood cultures were obtained in 464 cases, and that 152 of these (only 11 grew *Streptococcus pneumoniae*) were positive. However, no information was provided concerning the 141 non-pneumococci isolates. These data are of extreme importance, as hardly any data concerning bacteraemic community-acquired pneumonia are available. It was also reported that 112 of 464 patients were receiving antibiotic treatment when the blood cultures were inoculated. It would be interesting to know how many of these 112 blood cultures were positive and which pathogens were cultured.

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## REPLY FROM PROFESSOR REINERT

Our prospective population-based survey [1] of invasive pneumococcal disease (IPD) among adults in North-Rhine Westphalia, Germany, included 202 of the 386 hospitals in the region, and the 27 microbiological laboratories that submitted reports of IPD in these hospitals to the National Reference Centre for Streptococci (NRCS). In addition, the degree of under-reporting in this region was evaluated. All 27 laboratories were asked to provide complete laboratory records on all cases of IPD, and 16 were able to do so. Data concerning all IPD isolates sent by each of these 16 laboratories to the NRCS were linked to the databases of each laboratory's information system. Moreover, in two additional studies, the frequency of obtaining blood cultures, as well as the incidence of previous antibiotic therapy, was analysed in patients with community-acquired pneumonia (CAP). For the year 2000, the frequency of obtaining blood cultures was determined for patients admitted to three university hospitals (Aachen, Düsseldorf and Cologne) with CAP. In addition, in 2001, the frequency of antibiotic treatment before blood cultures were obtained from CAP patients was determined by a detailed analysis of patient histories. As outlined in our paper in *CMI* [1], all cases with the diagnosis of CAP (all diagnostic positions on the discharge summary) were identified *retrospectively* on the basis of ICD-10 codes (J13, J15.9, J18.0, J18.9). Cases were linked with data in the laboratory information systems of each hospital's microbiological laboratory.

Outcome data were obtained for all patients from the discharge files of the individual hospitals. Therefore, we are not able to provide the prospective data requested by Professor Shah. However, we would like to share the retrospective data for the 152 cases with positive blood